

AD-A086 136

TEXAS UNIV HEALTH SCIENCE CENTER AT DALLAS
ACTIVATION OF MEMBRANE-BOUND KALLIKREIN AND RENIN IN THE KIDNEY--ETC(U)
MAY 80 K NISHIMURA, F ALHENC-SELAS, A WHITE N00014-75-C-0807
UNCLASSIFIED TR-22 NL

1 OF 1
AD-A086 136

END
DATE
FILMED
8-80
DTIC

ADA 086136

LEVEL

(12)

OFFICE OF NAVAL RESEARCH

Contract N00014-75-C-0807

Technical Report No. 22

Activation of Membrane-bound Kallikrein and
Renin in the Kidney

Prepared by

Ervin G. Erdős

For Publication in
Proceedings of National Academy of Sciences

Departments of Pharmacology and Internal Medicine
University of Texas Health Science Center at Dallas
5323 Harry Hines Boulevard
Dallas, Texas 75235

23 May 1980

Reproduction in whole or in part is permitted for
any purpose of the United States Government

Distribution of this report is unlimited

DDC FILE COPY

DTIC
ELECTE
JUL 1 1980
S D
A

80 7 1 069

OFFICE OF NAVAL RESEARCH

Contract N00014-75-C-0807

Technical Report No. 22

Activation of Membrane-bound Kallikrein and
Renin in the Kidney

Prepared by

Ervin G. Erdős

For Publication in
Proceedings of National Academy of Sciences

Departments of Pharmacology and Internal Medicine
University of Texas Health Science Center at Dallas
5323 Harry Hines Boulevard
Dallas, Texas 75235

23 May 1980

Reproduction in whole or in part is permitted for
any purpose of the United States Government

Distribution of this report is unlimited

Accession For	
NTIS GRA&I	<input checked="checked" type="checkbox"/>
DDC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or special
A	I

REPORT DOCUMENTATION PAGE		REPORTING OFFICE NAME AND ADDRESS	
1. REPORT NUMBER Technical Report No. 22 /	2. GOVT ACCESSION NO. AD-A086136	3. REPORTING OFFICE NAME AND ADDRESS	4. REPORTING OFFICE ADDRESS
5. TITLE (and Subtitle) ACTIVATION OF MEMBRANE-BOUND KALLIKREIN AND RENIN IN THE KIDNEY		6. TYPE OF REPORT & PERIOD COVERED Technical 5/15/79 to 8/15/80	
7. AUTHOR(s) Nishimura, K., Alhenc-Gelas, F., White, A. and Erdős, E.G.		8. CONTRACT OR GRANT NUMBER N00014-75-C-0807	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Univ. Texas Health Science Center at Dallas Department of Pharmacology 5323 Harry Hines Blvd. Dallas, Texas 75235		10. REPORTING OFFICE PROJECT, TASK AREA & WORK UNIT NUMBERS NR 105-785	
11. CONTROLLING OFFICE NAME AND ADDRESS Office of Naval Research, Biol. & Med. Sci. Div. Medical and Dental Sciences Program, Code 444, Arlington, Virginia		12. REPORT DATE 5/23/80	
13. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		14. NUMBER OF PAGES 22	
15. SECURITY CLASSIFICATION (of this report) Unclassified		16. DECLASSIFICATION/DOWNGRADING SCHEDULE	
17. DISTRIBUTION STATEMENT (of this Report) Distribution of this document is unlimited			
18. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)			
19. SUPPLEMENTARY NOTES Proceedings of To be published in National Academy of Sciences			
20. KEY WORDS (Continue on reverse side if necessary and identify by block number) melittin/ lysolecithin/ aldosterone/ angiotensin/ kinins			
21. ABSTRACT (Continue on reverse side if necessary and identify by block number) Rat kidney contains membrane-bound renin and kallikrein. Kallikrein activity was measured with a spectrophotometric assay and renin by radioimmunoassay. Plasma membrane-bound kallikrein was activated by lysolecithin and also by melittin, which was a more potent activator. This activation by melittin was independent of calcium concentration. Melittin was, however, a more potent activator of bound-renin in the presence of calcium. Administration of aldosterone to rats for six days increased kallikrein activity in the renal homogenate and in the			

DD FORM 1 JAN 73 1473

EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-014-6601

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

↓ membrane-enriched fractions, while renin activity was not affected.
It was proposed that kallikrein may be located also on the basal
membrane of tubular epithelial cells, where aldosterone can enhance
its activity. ↙

ACTIVATION OF MEMBRANE-BOUND KALLIKREIN AND RENIN
IN THE KIDNEY

Key Words: (melittin/lysolecithin/aldosterone/angiotensin/
kinins)

Nishimura, K., Alhenc-Gelas, F., White, A. and Erdös, E.G.
Depts. of Pharmacology and Internal Medicine
University of Texas Health-Science Center at Dallas
5323 Harry Hines Boulevard, Dallas, Texas 75235

Abbreviations Footnote:

PM = plasma membrane ER = endoplasmic reticulum
S-2266 = D-Val-Leu-Arg-p-nitroanilide
Ang I = angiotensin I

ABSTRACT

Rat kidney contains membrane-bound renin and kallikrein. Kallikrein activity was measured with a spectrophotometric assay and renin by radioimmunoassay. Plasma membrane-bound kallikrein was activated by lysolecithin and also by melittin, which was a more potent activator. This activation by melittin was independent of calcium concentration. Melittin was, however, a more potent activator of bound-renin in the presence of calcium. Administration of aldosterone to rats for six days increased kallikrein activity in the renal homogenate and in the membrane-enriched fractions, while renin activity was not affected. It was proposed that kallikrein may be located also on the basal membrane of tubular epithelial cells, where aldosterone can enhance its activity.

The excretion of kallikrein in urine has been investigated in numerous laboratory and clinical conditions connected with hypertension (1-3). Although urinary kallikrein originates from the kidney (1-7), little is known about the biosynthesis, mode of activation and release of renal kallikrein. Kallikrein can be released into the urine from the distal tubules (7), but is absent from the brush border of the proximal tubules (8). Particulate fractions of the homogenized rat kidney enriched in plasma membranes (PM) contain both bound kallikrein (8-10) and renin (9, 11), and both kallikrein and renin are also found in fractions enriched in endoplasmic reticulum (ER) (8, 11). The finding that the kidney contains potent hypotensive and hypertensive enzymes bound to membranes led us to study the mode of activation of kallikrein and renin in fractions isolated from the rat kidney. We have also investigated how the sodium metabolism regulating hormone aldosterone affects the activity of kallikrein and renin in the kidney, and found that aldosterone increased membrane-bound kallikrein activity.

MATERIALS AND METHODS

Dextran T 70 was purchased from Pharmacia Inc. Piscataway, NJ. D-Val-Leu-Arg-p-nitroanilide (S-2266) was obtained from Kabi Diagnostica, Stockholm, Sweden. Melittin, d-aldosterone-21 acetate and other reagents were from Sigma Chemical Co., St. Louis, MO. Lysolecithin was purchased from Supelco, Inc., Bellefonte, PA. Synthetic melittin was obtained from Professor L. Levine of Brandeis University.

Enzyme assays

Kallikrein activity was assayed with S-2266 substrate. The hydrolysis of S-2266 was determined in a Cary 118 spectrophotometer, either by continuously recording the increase in absorption due to p-nitroaniline release at 405 nm, or by taking aliquots from the incubation mixtures at regular time intervals (10-12). An 0.1 M Hepes buffer, pH 9.1, was used at 37°C. With turbid solutions the following technique was employed: the reaction was stopped with 20% (w/w) perchloric acid (300 μ l) added to the supernatant fluid (500 μ l). The solution was kept at 4°C for 10 min to diazotise and 0.5% (w/v) ammonium sulphamate (500 μ l) was added to destroy the excess nitrite; subsequently 0.05% naphthylethylenediamine dissolved in methanol (1 ml) was added. After the solution was kept at 37°C in the dark for 30 min, the absorbance was measured at the wave length of 546 nm. Activity was expressed as nmol of p-nitroaniline product formed per min from S-2266. Rat urinary

kallikrein activity was expressed in units (U). One U of kallikrein activity equals 1 μ mol of substrate cleaved in 1 min. Renin activity was expressed in ng or μ g of angiotensin I (Ang I) released from nephrectomized rat serum (11). If not otherwise indicated or when no activators were used, 1% Triton X-100 was added to both the renin and kallikrein preparations. Protein concentration was measured by the method of Lowry et al., with bovine serum albumin as a standard (13).

Fractionation

Male Sprague-Dawley rats (200-250 g) were killed by decapitation. Twenty g of renal cortex, dissected from eight to ten rats, was pooled and minced. Renal tissue was suspended in 200 ml of 10 mM Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose and homogenized in a Dounce homogenizer. Two ml of 0.1 M EDTA was added and the homogenate centrifuged at 10,000 g and at 30,000 g for 15 min. The supernatant containing microsomal particles was centrifuged again at 100,000 g for 60 min in a Beckman L5-65 ultracentrifuge. The microsomal pellet was suspended in 10 mM Tris-HCl buffer, pH 8.6, and recentrifuged at the above speed, then the pellet was resuspended in 1 mM Tris-HCl buffer, pH 8.6, and centrifuged again. The washed microsomal fraction was suspended in 50 ml of 1 mM $MgSO_4$ and 1 mM Tris-HCL buffer, pH 8.6, and dialyzed against the same buffer for 2 hr. The microsomal fraction was then separated into plasma membrane (PM) and endoplasmic reticulum (ER) enriched subfractions by layering it on

24 ml of 15% w/w dextran T 70 in 1 mM MgSO_4 and 1 mM Tris-HCl, pH 8.6, and centrifuging in a SW-27 swinging bucket rotor at 25,000 rpm for 16 h(8, 14). The PM containing fraction was concentrated at the interface and the pellet was rich in ER. Both fractions were resuspended in 10 mM Tris-HCl (pH 8.6) and MgSO_4 and centrifuged at 100,000 g for 60 min. The pellets were suspended in 10 mM TrisHCl (pH 7.4) containing 0.25 sucrose. The samples were kept at -70°C until used. There was no difference in kallikrein activity between the fresh samples and the samples stored frozen. Freshly collected fractions were used to assay bound renin.

Aldosterone

Sprague-Dawley rats in groups of six animals were injected s.c. with 0.25 mg aldosterone in 0.2 ml sesame oil for six days. The animals in the control group received only sesame oil. Urine samples were collected for 16 hr in metabolic cages. Blood samples were withdrawn from the tail vein. The kidneys of each animal in the control and in the treated group were homogenized, fractionated and assayed individually.

Activation of kallikrein

Aliquots of membrane-bound and urinary kallikrein used as control for soluble kallikrein were routinely preincubated with activators at 4°C for 30 min. In some experiments, melittin was directly added to the cell of the spectrophotometer containing

the substrate and enzyme without preincubation.

RESULTS

Kallikrein

Rat kidneys were homogenized, and separated into PM and ER enriched fractions by differential centrifugation as previously described (8, 10, 11). The separation procedure included repeated washings with hypotonic buffer. Kallikrein activity in the PM fraction (PM-kallikrein) averaged 1.81 nmol of S-2266 hydrolyzed per min by one mg protein prior to activation. As initial studies indicated that detergents (9-11), phospholipase A₂, lipids and the polypeptide melittin (11) enhance PM-kallikrein activity, we compared the effect of lysolecithin on PM-kallikrein with that of melittin. Fig. 1 shows that melittin was about a thousand times more active than lysolecithin on a molar basis.

Fig. 1

Lecithin and arachidonic acid were active only at a much higher concentration than lysolecithin. The melittin used in these studies was a natural product, but the synthetic peptide gave identical results. Since melittin activity is consistent with activation of endogenous phospholipase A₂ (15-17), we wished to determine whether the activation of PM-kallikrein is a direct or indirect effect of the peptide; we added an activator or an inhibitor of phospholipase A₂ to the PM-kallikrein melittin incubation mixture. Activation of PM-kallikrein by melittin in presence or absence of 1 mM CaCl₂, or 0.1 mM mepacrine or 1mM EDTA was

identical (not shown in Fig. 1). Possibly melittin has a direct action on the membrane; alternately the phospholipase is not activated by CaCl_2 or inhibited by mepacrine.

Renin

In addition to kallikrein, the PM enriched rat kidney fraction contained low renin activity (8-30 ng Ang I/hr per mg protein). This activity was enhanced by the activators of PM-kallikrein. Melittin activated bound renin in presence of added 1 mM CaCl_2 in the same concentration as kallikrein. However, the dose-response curve was shifted to the right when CaCl_2 was deleted or EDTA was added (Fig. 2) to the incubation mixture. For an equivalent activity in absence of calcium, the concentration of melittin had to be an order of magnitude higher. Fig. 2

Effect of aldosterone

Because aldosterone enhances the excretion of kallikrein in urine (1-3, 18, 19), we studied the effect of the hormone on kallikrein and renin in rats. In agreement with the findings of others, aldosterone increased the excretion of kallikrein after six days of administration from a mean of 2.4 ± 0.17 U in the urine collected in 16 hr to 4.4 ± 0.35 U ($p < .001$). Aldosterone also decreased the plasma renin activity considerably from 11.1 ± 0.96 to 3.8 ± 0.37 ng Ang I/h per ml ($p < .001$).

The activity of renin and kallikrein was also assayed in fractions of the homogenized kidneys of rats treated with aldosterone and of control animals.

Most of the renin activity in the kidney was, as expected, in the soluble fraction, but aldosterone had no significant effect

on the renin activity in the crude homogenate or in the particulate fraction. The activity of renin in the crude homogenate and in the washed particulate fraction in treated and untreated rats was 5.1 ± 0.4 , 4.9 ± 0.6 , 0.4 ± 0.06 and 0.5 ± 0.07 μ g of Ang I released per hr by 1 mg protein respectively.

In contrast to renin, administration of aldosterone increased kallikrein activity in the kidney (Table 1). The difference between the treated animals and the control groups became highly significant in the particulate fraction (100,000 g sediment). After washing and resedimenting, this fraction from control animals had an activity of 2.8 ± 0.3 nmol/min per mg while that from treated animals cleaved 6.4 ± 0.8 nmol of substrate ($p < 0.002$). Table 1

In a second series of experiments the kidneys were further fractionated to PM and ER enriched fractions. The activity of kallikrein in the PM fraction was 11.2 ± 2 nmol in the control and 23.3 ± 1.3 in the aldosterone group ($p < .001$). The corresponding figures were 2.1 ± 0.7 and 5.7 ± 0.5 in the ER fractions ($p < .002$). The variations in renin activity in the PM and ER fractions of the treated and untreated animals were within experimental error.

DISCUSSION

The importance of the renin-angiotensin system in the etiology of some forms of hypertension is undisputed, but the role of kallikrein and kinins in the pathogenesis of this disease is still being investigated. Excretion of kallikrein

in the urine is decreased in clinical and experimental hypertension and is increased by aldosterone (1-3, 18, 19). The level of this enzyme in urine is also influenced by fluid intake, diet, diuretics, and race (1-3). The actions of kinins in the kidney include vasodilation, increased sodium and water excretion (1, 2), activation of a phospholipase and release of prostaglandin (20). Most of the studies on urinary kallikrein were based on measuring its esterase activity, but the function of kallikreins is to cleave peptide bonds.

We determined the activity of renal kallikrein with the specific peptide substrate S-2266 (12). The activity of renal kallikrein, as measured by radioimmunoassay of the kinin released, correlated well with the rate of cleavage of S-2266, but not with arginine esterase activity (10, 11). Other investigators also concluded that changes in the urinary esterase activity do not necessarily reflect changes in the amount of active kallikrein excreted (21).

In the rat, the kidney contains most of the renin in the body. Renin is concentrated in the granules of the juxtaglomerular cells, where it is released into the circulation (22). However, renin also has intrarenal functions (23), that may call for a localization of the enzyme outside of the juxtaglomerular apparatus. We reported previously that renin can be induced in a particulate (microsomal-ribosomal) fraction of the submaxillary gland of mice

(24). In addition to marker enzymes and kallikrein (8, 11), the PM and ER enriched fractions of the rat kidney also have renin activity, even after repeated washing of the fractions with hypotonic solutions. Transmission electron micrographs of the preparation revealed no trapped granules or soluble material in the isolated membrane vesicles (11).

Bound kallikrein and renin in the PM and ER enriched fractions of the kidney are activated by various agents. These include phospholipase A_2 (10, 11) and melittin. Melittin is a polypeptide with a M_r of about 2800, which contains basic and hydrophobic amino acids. It interacts with membranes (25), and its activity is consistent with activation of phospholipase at low concentration (15-17). Phospholipase A_2 of pancreas or snake venom added to the membrane fractions (10, 11) or isolated kidney cells (26) enhanced kallikrein activity. Since a chloroform extract of the incubation mixture of phospholipase A_2 and a membrane fraction also activated PM-kallikrein, we tested various lipids as activators and found lysolecithin to be more potent than prostaglandin E_2 (11), arachidonic acid or lecithin. However, melittin, on a molar basis, was about three orders of magnitude more potent than lysolecithin (Fig. 1).

Experiments with melittin indicated that PM-renin and PM-kallikrein are activated by different mechanisms. Melittin's activity was not affected by $CaCl_2$, EDTA or mepacrine. Melittin may act by

activating a membrane-bound prekallikrein prior to releasing the enzyme into solution (9), or by activating a calcium-independent phospholipase. The activation of PM-renin by melittin was greatly enhanced by calcium and was decreased by EDTA (Fig. 2). This indicates that melittin released bound renin by a calcium dependent process, possibly by activating a calcium dependent phospholipase.

The administration of aldosterone to rats for six days influenced kallikrein and renin activity differently. Aldosterone treatment decreased plasma renin activity in the rats and increased the excretion of kallikrein in the urine. The hormone did not affect activity of renin in the kidney, but increased that of kallikrein. The difference between the treated animals and the control group became more significant after the membrane fractions were separated from the crude homogenate.

The relationship of kallikrein to mineralocorticoids has been studied extensively by Margolius and his colleagues and others (1-3, 18, 19, 27-29) who reported that aldosterone increases kallikrein excretion in man and animals. Margolius et al., hypothesized that aldosterone is an inducer of renal kallikrein (30). Our experiments showed that aldosterone enhances kallikrein but not renin activity in the kidney, presumably by inducing its synthesis in the microsomal-ribosomal fraction, which leads to increase in kallikrein on the membrane of the cells of the distal tubules.

We can only guess the exact site of action of aldosterone on renal kallikrein. Over 90% of renal kallikrein is in the cortex (1), where it can be released from the distal tubules (1-3, 7, 8). It has also been shown that kallikrein is located on the luminal surface of the distal tubules (31). However, special fixation of tissues was required before kallikrein activity could be shown by immunofluorescence. This is understandable because kallikrein bound to membrane reacts much less with antibody than the soluble kallikrein (11). A localization of kallikrein on the luminal surface, however, cannot explain all the actions of kallikrein on the kidney. For example, kallikrein was detected in venous effluent of the kidney (32) and in renal lymph (33). In addition, although kallikrein substrate, kininogen, was found in the urine (34), the site of interaction of kininogen with renal kallikrein has not yet been determined.

In secretory glands kallikrein is present in cells which are rich in mitochondria and contain infoldings of basal cell membranes, a structure usually associated with water and ion transport (35). The ultrastructure of the basal part of the epithelium in distal tubules, which face the peritubular capillaries, also shows concentrated mitochondria and infolding of cell membranes (36).

In the rabbit, corticosteroids acted on the basolateral membrane of the collecting tubules and enhanced its surface area, but did not affect the luminal membrane area (37).

Using isolated tubular segments of the rabbit kidney, Gross and Kokko (38) found that aldosterone stimulated base-line potential across cortical collecting ducts in vitro. In the rat, however, aldosterone modulated in vivo Na-K-ATPase activity in all renal tubular structures studied (39). If kallikrein and prekallikrein were also located on the basal membrane of the epithelial cells of the distal tubules, the role of kallikrein in renal vascular resistance (40, 41), its release into circulation (32) and extracellular fluid (33), would be easier to understand than if it were located only on the luminal rim of the tubular cells. Aldosterone may also enhance renal kallikrein activity by acting on the basal membrane of tubular cells.

Fig. 1. Activation of plasma membrane-bound kallikrein by arachidonic acid, lecithin, lysolecithin and melittin.
Abscissa: M concentration.
Ordinate: relative rate of hydrolysis of S-2266.

Fig. 2. Activation of plasma membrane-bound renin by melittin in presence of 1mM CaCl_2 Δ — Δ , in absence of added CaCl_2 \square — \square and in presence of 10 mM EDTA \circ — \circ .
Abscissa: M concentration.
Ordinate: ng Ang I released per hr in 1 ml.

ACTIVATION OF PM MEMBRANE-BOUND KALLIKREIN

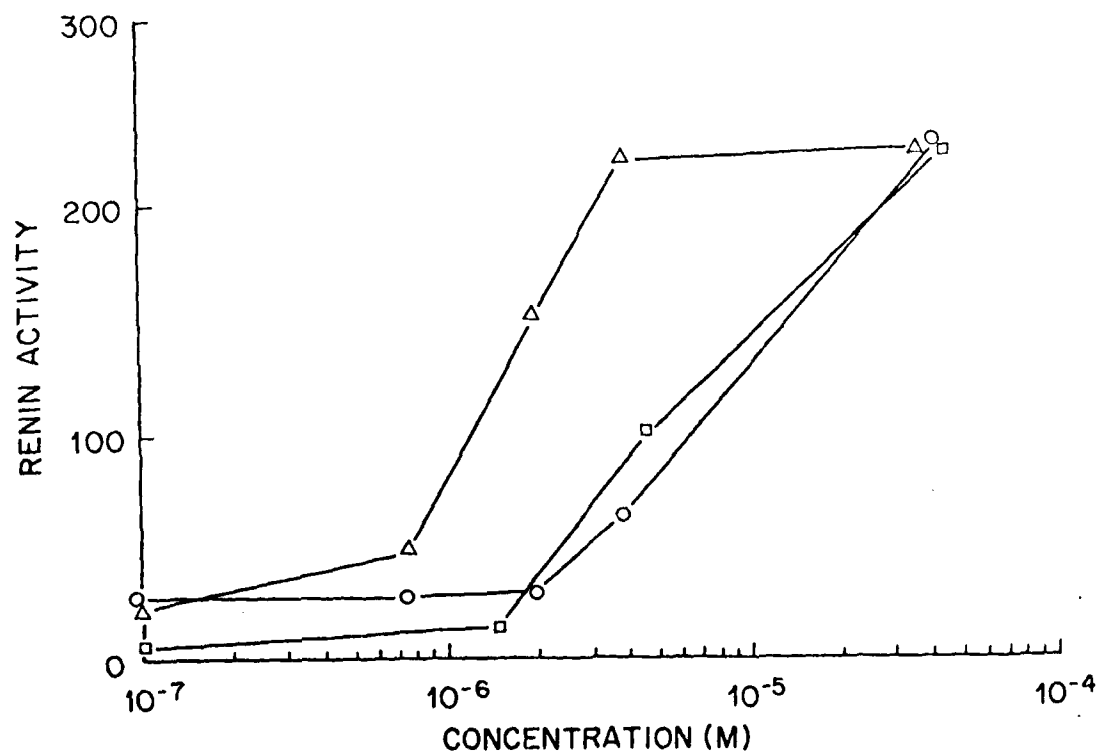
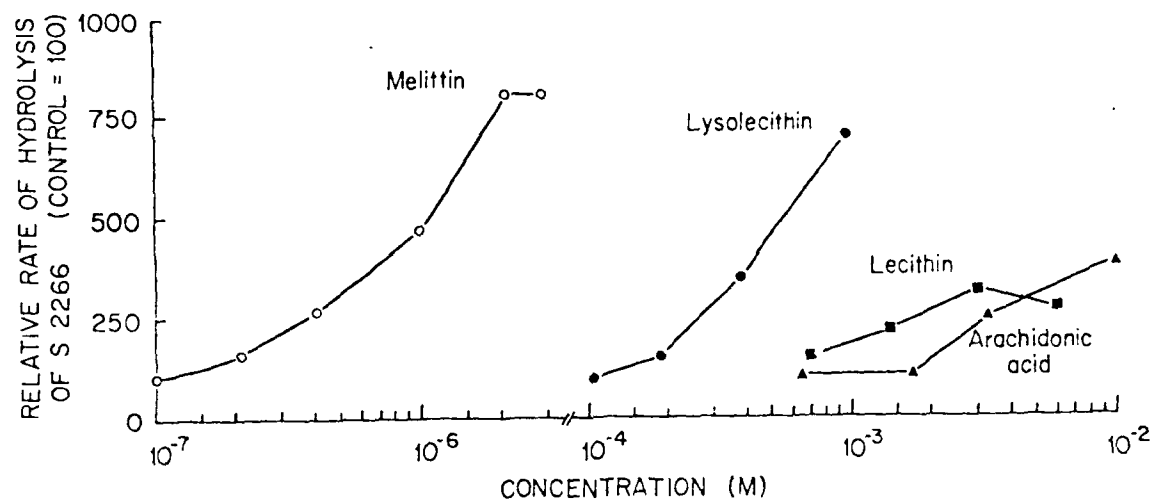


TABLE 1

EFFECT OF ADMINISTRATION OF ALDOSTERONE
ON THE ACTIVITY OF KALLIKREIN IN RAT KIDNEY

	Control		Aldosterone		
	1	2	1	2	
Crude homogenate	0.6 ± 0.06	1	0.8 ± 0.16	1	$p = .058$
High speed pellet	1.92 ± 0.2	3.3	4.6 ± 0.6	5.8	$p < .022$
Washed particulate fraction	2.8 ± 0.3	4.3	6.4 ± 0.8	8	$p < .002$

1. Specific activity nmol/min/mg \pm S. E.

2. Relative specific activity, enrichment factor.

1. Carretero, O.A. & Scicli, A.G. (1978) *Klin Wochenschrift* 56, Suppl. 1, 113-125.
2. Ward, P.E. & Margolius, H.S. (1979) in *Handbook of Exper. Pharmacology*, ed. Erdős, E.G. (Springer-Verlag, Heidelberg) XXV Suppl., pp. 525-548.
3. Mills, I.H. (1979) in *Handbook of Exper. Pharmacology*, ed. Erdős, E.G. (Springer-Verlag, Heidelberg) XXV Suppl., pp. 549-567.
4. Werle, E. (1960) in *Polypeptides which affect smooth muscles and blood vessels*, ed. Schachter, M. (Pergamon Press, Oxford), pp. 199-209.
5. Carvalho, I.F. & Diniz, C.R. (1966) *Biochim. Biophys. Acta* 128, 136-148.
6. Nustad, K. (1970) *Brit. J. Pharmacol.* 39, 73-86.
7. Scicli, A.G., Carretero, O.A., Hampton, A., Cortes, P. & Oza, N.B. (1976) *Amer. J. Physiol.* 230, 533-536.
8. Ward, P.E., Erdős, E.G., Gedney, C.D., Dowben, R.M. & Reynolds, R.C. (1976) *Biochem. J.* 157, 643-650.
9. Wilson, C.M., Ward, P.E., Erdős, E.G. & Gecse, A. (1976) *Circul. Res.* 38, 1195-1198.

10. Nishimura, K. & Erdös, E.G. (In Press) 8th Workshop Conference, Hoechst Enzymatic Release of Vasoactive Peptides. eds. Gross, F., Vogel, G. (Raven Press, New York).
11. Nishimura, K., Ward, P.E. & Erdös, E.G. (In Press) Hypertension.
12. Amundsen, E., Putter, J., Friberger, P., Knos, M., Larsbraten, M. & Claeson, G. (1979) in Kinins-II: Biochemistry, Pathophysiology and Clinical Aspects, eds. Fujii, S., Moriya, H. & Suzuki, T. (Plenum, New York) pp. 83-95.
13. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
14. Wallach, D.F.H. & Kamat, V.B. (1966) Methods in Enzymology 8, 164-172.
15. Tashjian, Jr., A.H., Ivey, J.L., Delclos, B. & Levine, L. (1978) Prostaglandins 16, 221-232.
16. Hassid, A. & Levine, L. (1977) Res. Com. Chem. Path. Pharmacol. 18, 507-517.
17. Shier, W.T. (1979) Proc. Natl. Acad. Sci. USA 76, 195-199.
18. Margolius, H.S., Horwitz, D., Geller, R.G., Alexander, R.W., Gill, Jr., J.R., Pisano, J.J. & Keiser, H.R. (1974) Circ. Res. 35, 812-819.

19. Vinci, J.M., Zusman, R.M., Izzo, Jr., J.L., Bowden, R.E., Horwitz, D., Pisano, J.J. & Keiser, H.R. (1979) *Circ. Res.* 44, 228-237.
20. Terragno, N.A. and Terragno, A. (1979) in *Handbook of Exper. Pharmacology* ed. Erdös, E.G. (Springer-Verlag, Heidelberg) XXV Suppl., pp. 401-426.
21. Carretero, O.A., Amin, V.M., Ocholik, T., Scicli, A.G. & Koch, J. (1978) *Circ. Res.* 42, 727-731.
22. Davis, J.O., Freeman, R.J. (1976) *Physiol. Rev.* 56, 1-56.
23. Vecsei, P., Hackenthal, E. and Ganten, D. (1978) in *Klin. Wochenschrift* 56, Suppl. 1, 5-21.
24. Gecse, A., Wilson, C.M. & Erdös, E.G. (1976) *Biochem. Pharmacol.* 25, 763.
25. Habermann, E. (1972) *Science* 177, 314.
26. Chao J., & Margolius, H.S. (1979) *Biochem. Biophys. Acta* 570, 330-340.
27. Geller, R.G., Margolius, H.S., Pisano, J.J. & Keiser, H.R. (1972) *Circ. Res.* 31, 857-861.
28. Margolius, H.S., Horwitz, D., Pisano, J.J. & Keiser, H.R. (1976) *Fed.*

Proc. 35, 203-206.

29. Nasjletti, A., McGiff, J.C. & Colina-Chourio, J. (1978) *Circ. Res.* 43, 799-807.
30. Margolius, H.S., Chao, J. & Kaizu, T. (1976) *Clin. Sci. Molecular Med.* 51, 279s-282s.
31. Ørstavik, T.B., Nustad, K., Brandtzaeg, P. & Pierce, J.V. (1976) *J. Histochem.* 24, 1037-1039.
32. Roblero, J., Croxatto, H., Garcia, R., Corthorn, J. & De Vito, E. (1976) *Amer. J. Physiol.* 231, 1383-1389.
33. Mills, I.H., MacFarlane, N.A.A., Ward, P.E. & Obika, L.F.O. (1976) *Fed. Proc.* 35, 181-188.
34. Pisano, J.J., Corthorn, J., Yates, K. & Pierce, J.V. (1978) In *Contributions to Nephrology*, eds. Eisenbach, G.M., Brod, J. (Basel, Karger) Vol. 12, pp. 116-224.
35. Ørstavik, T.B., Nustad, K. & Gautvik, K.M. (1979) in *Kinins II: Biochemistry, Pathophysiology and Clinical Aspects*, eds. Fujii, S., Moriya, H. & Suzuki, T. (Plenum, New York) pp. 439-450.
36. Rhodin, A.G. (1974) *Histology*, (Oxford Press, New York) p. 660.

37. Wade, J.B., O'Neil, R.G., Pryor, J.L. & Boulpaep, E.L. (1979)
J. Cell Biol. 81, 439-445.
38. Gross, J.B. & Kokko, J.P. (1977) J. Clin. Invest. 59, 82-89.
39. Schmidt, U., Schmid, J., Schmid, H. & Dubach, U.C. (1975)
J. Clin. Invest. 55, 655-660.
40. Levy, S.B., Lilley, J.J., Frigon, R.P. & Stone, R.A. (1977)
J. Clin. Invest. 60, 129-133.
41. Levinsky, N.G. (1979) Circ. Res. 44, 441-451.

ACKNOWLEDGEMENTS

The authors are grateful for the advice of Drs. J. P. Kokko and D. Marver of the Dept. of Int. Med. and for the consistent support and guidance of Dr. R. C. Reynolds, Dept. of Pathol. D. Page assisted skillfully in these studies.

These studies were supported in part by Grants NIH HL 20594, NIH HL 16320, NIH HL 14187 and ONR N00014-75-C-0807.